DEVELOPMENT OF PRETREATMENT TECHNIQUES FOR HIGH PRODUCTIVITY GAHARU EXTRACTION

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ABSTRACT

Gaharu or Agarwood is a resinous wood from aquilaria species, naturally grown in tropical forest. It is of high demand from various industries for it contains therapheutical essential oil commonly used in cosmetic industry, religious ceremony and traditional medicine. Due to its high demand and scarce in the forest, gaharu oil is highly priced. As of early 2010, gaharu oil in Malaysia is valued at RM420 per tola (12g). Hence, it is of great importance for an efficient method of extracting the oil to be developed. The conventional method of extraction currently practiced in the industry requires very long hours and produces low oil yield. The present study focused on developing pretreatment steps of conditioning the gaharu wood prior to extraction in order to enhance the extraction process. Preliminary experimental work showed that all pretreatment methods of gaharu wood examined in the study strongly influenced the oil yield during extraction. Four types of pretreatment methods were examined, namely soaking (typically used in industry), ultrasonication, enzymatic and combination of ultrasonication and enzymatic. From the study, combination of ultrasonication and enzymatic pretreatment method was found to give the highest oil yield (0.1232%), followed by ultrasonication (0.1134%), enzymatic (0.1088%) and soaking (0.0734%) respectively. In comparison to untreated sample, an improvement of 53.57% was achieved in the extraction of sample pretreated with combination of ultrasound and enzymatic. On the other hand, soaking technique improved the oil yield by 28.32%. In general, hydro distillation performed better than steam distillation for extraction of gaharu oil, with extraction yield increased up to 35% due to the fact that hydro distillation provides continuous water phase within the solid structure as compared to steam distillation where not all solid surfaces are in contact with the passing steam. At optimum operating conditions, the highest oil yield from combination of ultrasonic and enzymatic pretreatment method was 0.1692%, at 9 hours pretreatment time, 1:16w/v of sample to water ratio and 1.5:100v/w of enzyme to substrate ratio. Calculation of extraction rate constant (K_o) showed that K_o for the combination of ultrasonic and enzymatic pretreated sample was greater than the value for the conventional (soaking) pretreated sample, with value of 0.45 and 0.25 respectively. The increased of K_o value indicates greater driving force of mass transfer at the solid-liquid interface.

ABSTRAK

Gaharu atau *Agarwood* adalah sejenis kayu yang beresin dari spesis aqualaria yang tumbuh secara semulajadi di hutan tropika. Gaharu mendapat permintaan yang tinggi kerana mengandungi pati minyak terapi yang kebanyakan digunakan dalam industri kosmetik, upacara keagamaan dan sebagai ubatan tradisional. Permintaan yang tinggi dan sukar diperoleh dari hutan menyebabkan harganya sangat mahal. Sehingga awal tahun 2010, harga minyak gaharu di Malaysia mencecah RM420 untuk setiap tola (12g). Oleh itu, kaedah mengekstrak minyak secara berkesan adalah amat penting untuk dibangunkan. Kaedah konvensional yang digunakan dalam industri sekarang ini memerlukan masa pengekstrakan yang lama dan menghasilkan minyak yang sangat sedikit. Kajian ini memfokuskan langkah pra-rawatan sebelum proses pengekstrakan kayu gaharu untuk meningkatkan kadar pengekstrakan. Keputusan awal eksperimen menunjukkan semua kaedah pra-rawatan yang diuji amat mempengaruhi penghasilan minyak semasa proses pengekstrakan. Empat jenis kaedah pra-rawatan telah diuji iaitu rendaman (digunakan dalam industri sekarang), ultrasonik, enzimatik dan kombinasi ultrasonik dan enzimatik. Keputusan ujian menunjukkan kaedah pra-rawatan kombinasi ini menghasilkan minyak yang paling banyak (0.1232%), diikuti oleh ultrasonik (0.1134%), enzimatik (0.1088%) dan rendaman (0.0734%). Berbanding dengan sampel yang tidak melalui pra-rawatan, sebanyak 53.57% peningkatan telah dicapai dengan pengekstrakan sampel yang melalui pra-rawatan kombinasi ultrasonik dan enzimatik. Manakala peningkatan sebanyak 28.32% dengan teknik pra-rawatan rendaman. Secara general, penyulingan hidro adalah lebih baik berbanding penyulingan stim bagi pengekstrakan minyak gaharu, dengan perbezaan penghasilan sehingga 35%. Hal ini disebabkan oleh penyulingan hidro membekalkan fasa air yang berterusan di antara struktur pepejal berbanding dengan penyulingan stim, iaitu tidak semua pepejal bersentuhan dengan aliran stim semasa proses pengekstrakan. Pada keadaan operasi yang optimum, penghasilan minyak paling tinggi ialah pengekstrakan sampel yang melalui kaedah pra-rawatan kombinasi ultrasonik dan enzimatik iaitu 0.1692%, dengan 9 jam tempoh pra-rawatan, 1:16w/v kadar sampel terhadap air dan 1.5:100v/w kadar enzim terhadap sampel. Kiraan kadar pengekstrakan tetap (K_o) menunjukkan bahawa K_o untuk sampel kombinasi pra-rawatan ultrasonik dan enzimatik adalah lebih besar daripada nilai untuk sampel konvensional (rendaman) dengan nilai masing-masing ialah 0.45 dan 0.25. Peningkatan nilai K_o menunjukkan peningkatan kadar aliran pergerakan bendalir di permukaan pepejal-cecair.

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c	Concentration
C	Sound velocity
cA	Concentration of A
c_{L1}	Bulk fluid concentration
c_{Li}	Concentrationj in the fluid next to the surface of the solid
c_p	Specific heat at constant pressure
c_t	Concentration at time t
c_∞	Concentration at equilibrium
c_v	Specific heat
D	Diffusion
D_{AB}	Molecular diffusivity of the molecule A and B
D_{ac}	Diffusion coeffiecient in the sound field
E	Bulk modulus of elasticity
E_k	Kinetik energy
E_p	Potential energy
I_o	Intensity of the sound wave
J_{*AZ}	Molar flux of component A in the z direction
k_c	Mass transfer coefficient
K_o	Extraction rate constant
N_A	Rate of convective mass transfer
p	Sound pressure
$\mathrm{P_a}$	Acoustic pressure
$\mathrm{P_h}$	Normal atmospheric pressure
$\mathrm{P_L}$	Total liquid pressure
T	Temperature
u	Velocity of displacement
v	Viscosity
V	Acoustic particle velocity amplitude
V_o	Steady state volume
x_A	Mole fraction of A
x	Path traversed by the sound wave
β^*	Thermal expansion coefficient
ε_M	Turbulent or mass eddy diffusivity
η	Dynamic viscosity coefficient
ρ	Density of the medium
ω	Cyclic frequency of the sound wave

CHAPTER 1

INTRODUCTION

1.1 Research Background

Aqualaria malaccensis or gaharu is a resin impregnated of Aqualaria species. This wood is able to produce unique aromatic scent and categorized as one of the most expensive and highly prized commodities. Several compound such as agarospirol, jinkohol-eremol and kusenol have been reported to possibly contribute to the characteristic aroma of gaharu (Nakanishi et al., 1984; Ishihara et al., 1993).

Gaharu is divided into several grades in the market such as super grade A, grade A, B, C and D. The higher quality gaharu wood can be recognized by its dark colour and strong aroma released upon burning its chips or quality incense. However, there is very little information on the quality of different grade gaharu essential oils produced. The best and darkest gaharu woods are used in incense mixtures while the lower grades are extracted to produce gaharu oil used in perfumery. On average, the oil represents 1% of the total weight of the lower grades gaharu wood (Chang et al., 2002).

Gaharu is traditionally used to produce incense for rituals and religious ceremonies in the Far East. Gaharu is also believed to have tonic and therapeutic properties (Burkill, 1966). In Asia, gaharu is used to treat smallpox, rheumatism, to heal wound and illness during and after birth

Gaharu essential oil is high in demand in the perfume industry as evidenced by the recent expansion of the range of uses for gaharu. These include products such as gaharu essence, soap and shampoo (Chakrabakty et al., 1994). These products have been marketed at prices about ten times more expensive than the common brands of toilet soaps and shampoos. With advancing technology, it is expected that in future more new products that derives from gaharu will appear in the market.

Currently, other uses of this product are restricted and limited due to its rarity and high prices. As May 2009, the price is around RM 420 per 12 grams (Fatimi, 2009) and others report RM65 000 per liter for lower grades and superior grades could be priced up to RM 150 000 per liter (http://usahawangaharu.blogspot.com).

It is anticipated that the prices of gaharu will remain high in the future because of the high demand for gaharu material in Middle East countries. Introduction of new applications for gaharu materials in cosmetic industry and the traditional uses of gaharu in China, Japan and India for manufacturing of joss-sticks and other products (Chang et al., 2002), given tremendous demand and diverse applications of gaharu, the economic potential of this product is substantial.

1.2 Problem Statement

Common extraction method of gaharu essential oil is via conventional hydro distillation but nowadays the extraction of essential oils from plant material can be achieved by a number of different methods. The choice of extraction method will depend on the nature of the material, the stability of its chemical components and the specification of the targeted product. The commonly used methods of extracting essential oil from plant material include hydro distillation, steam distillation and solvent extraction/leaching process.

Commercially in Malaysia, gaharu oil is obtained by hydro distillation process. The main advantage of hydro distillation is that it can be generally carried out with very simple equipment. Using distillation method, the plant material is mixed directly with water in a still pot. A perforated grid is inserted above the base of the still pot to prevent the plant material settling on the bottom and coming in direct contact with the heated base of the still pot and causing it to char.

Current commercial method involved long duration of extraction (5-15 days), hence ineffective of energy usage. Thus, need improvement to give better quality, yield and less energy usage to make the business more attractive. Explore alternative or other method of extraction to see its effectiveness in extracting gaharu oil. Figures 1.1 and 1.2 show the conventional extraction method (distillation process) which could not extract and separate the oil from solid particle very effectively. Thus, improvement on gaharu oil extraction method should be carried out in order to get a better quality and quantity of the gaharu oil yield.

It is expected that through understanding gaharu oil extraction mechanism, there will be sufficient supply to meet market demands. Therefore, there is a strong incentive to maximize gaharu oil extraction yield and one of the challenges in commercializing gaharu in Peninsular Malaysia is to produce high quality gaharu oil (Mohd Paiz, 2006).

Figure 1.1: Conventional method of gaharu extraction

Figure 1.2: The remains oil of gaharu that can not be separated effectively

1.3 Objective of Research

The objectives of this study are to:-

i) examine the feasibility of the pretreatment process on gaharu extraction via hydro distillation process on ground gaharu.

ii) identify the most influential operational parameters in the pretreatment process that affect the percentage of oil collected in the process.

iii) extract gaharu oil using appropriate extraction method for better gaharu oil yield.

1.4 Scope of the Research

In order to achieve the objectives, the following scopes have been identified:

1. Study and investigate the various pretreatment processes via hydro distillation process under similar condition.
2. Evaluate the findings to determine the reliability of pretreatment process as a new technique of gaharu oil extraction.
3. Study the various operating conditions on the extraction of gaharu oil. The main variables investigated are:
   i. the effects of several types of pretreatment process on the gaharu oil yield extraction.
   ii. the effects of pretreatment time.
   iii. the effect of sample to water ratio during extraction process of gaharu oil.
4. To compare the performance of hydro distillation and steam distillation extraction techniques in extracting gaharu oil.
5. Study the extraction rate constant in comparing conventional pretreatment method and the selected (highest yield) pretreatment method.
6. Comparing the analysis of gaharu essential oil compound for untreated and pretreated samples.

1.5 Importance of Research

Several extraction methods have been conducted such as hydro distillation, clavenger and solvent extraction which are carried out at different process scale. The oil was successfully extracted using these methods, but there is still need to improve the process in terms of extraction time, yield and quality of oil. The preliminary result concluded from conventional hydro distillation method revealed that agarospirol (13.49%) and selin-4,7 (11)-diene (13.11%) are the major components of reddish brown oil, meanwhile greenish brown oil consists of delta-selinene (11.11%) and rotundone (8.37%). The quality of gaharu oil using hydro distillation is better than solvent extraction. Besides, pretreatment was used to shorten the process time and the gaharu oil can be released easier in the next process sequence. The aim of this study is to help gaharu oil entrepreneur to use better technology to increase their revenue and for the country to be a leader in gaharu product.

This study is an alternative and improvement for extraction of gaharu oil which can reduce the extraction time and increase the oil yield using distillation method. Prior to that, it must undergo the process of cell wall breakage as the pretreatment steps.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction of Gaharu

Gaharu or agarwood is the resinous wood from the aquilaria tree and evergreen native to northern India, Laos, Cambodia, Malaysia, Indonesia and Vietnam. Its scientific name is *Aquilaria Malaccensis,* in the family of Thymelaeaceae. Other common names for gaharu are Aloeswood, Agarwood, Jin Koh, Jinko, Eagle wood, Oud, and Ood ud.

Gaharu tree (Figure 2.1) has adapted to live in various habitats, including those that are rocky, sandy or calcareous, well-drained slopes, ridges and land near swamps. It can grow up to 40 meters high and 60 centimeters trunk diameter (Chang et al., 2002). The average diameter growth rate in Malaysia forest is rather low at 0.33 cm/ year, and the fastest-growing larger specimens are reported to grow at 0.8-1 cm/year.

Formation of gaharu oil occurs in the trunk and roots of trees that have been infected by fungus (Figure 2.2). As a response, the tree produces a resin high in volatile organic compounds that aids in suppressing or retarding fungal growth. While the unaffected wood of the tree is relatively light in colour, the resin dramatically increases the mass and density of the affected wood, changing its colour from pale beige to dark brown or black. Other factors such as the age of the tree, differences in the tree caused by seasonal variation, environmental variation and genetic variation of *Aquilaria* may also play an important role in gaharu formation (Ng et al., 1997). Figure 2.3 shows the cross section of gaharu wood.

Figure 2.1: Gaharu tree

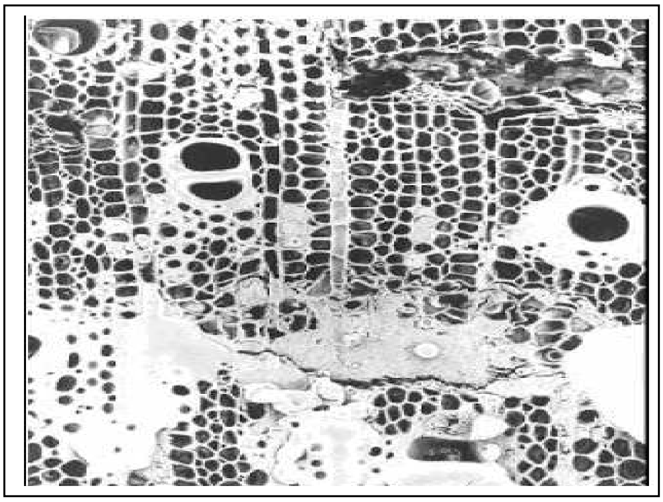

Figure 2.2: Cell structure within a gaharu tree, white areas indicate resin deposits

Source: Eriksson et al., 1990

Figure 2.3: Cross section of gaharu wood

Source: http://forestpathology.cfans.umn.edu

Gaharu in the form of chips (Figure 2.4 and Figure 2.5), oil and powder waste after extraction are the most common forms traded. In Malaysia there are records of the use of gaharu in various folk remedies for the treatment of weakness, stomach pains, in pregnancy, after delivery, fever, chest pains, body pains, rheumatism, women diseases and dropsy (Chang, et al., 2002). A decoction of the wood is used for abdominal pain, asthma, cancer, colic chest, congestion, diarrhea, hiccups, nausea and also regurgitation.

Figure 2.4: Low grade gaharu chip for extraction of essential oil

Figure 2.5: High quality gaharu chip

In the Muslim societies of the Middle East, oud (Arabic word for wood) is a symbol of status, wealth and hospitality. Gaharu incense is used in religious ceremonies by Buddhists, Hindus and Muslims, while a revival of the ‘Koh doh’ incense ceremony in Japan has rekindled interest in gaharu in that country.

Gaharu is graded in 5 classes, namely Super grade A, grade A, B, C, and D. Super grade A is the most expensive compared to the others. The grades are according to the physical properties, gaharu formation and its unique scent (Nor Azah et al., 2008). However, there is no existing standard method for grading of gaharu yet.

The lower grades such as Grade C are often distilled to obtain gaharu oils. Grade C gaharu wood were obtained from different sources mainly from Gua Musang, Kelantan, Kuala Terengganu, Terengganu, Gombak, Selangor and Merapoh, Pahang (Nor Azah et al., 2008). As noted in Barden et al. (2000), grading gaharu is a complicated process, includes evaluating the size, colour, odour, weight (on scales and in water) and flammability of the wood. However, application of grade codes (Super A, A, B, C, D, E) varies between buyers in Papua New Guinea (Frank and James, 2001).

2.1.1 Types of Gaharu

Five species of *Aquilaria* or gaharu are recorded for Peninsular Malaysia and all are believed to be able to produce oleoresins (Chang et al., 2002) as shown in Table 2.1. *Aquilaria malaccensis* is well distributed throughout Peninsular Malaysia, except for the states of Kedah and Perlis. It is confined mainly to plains, hill slopes and ridges up to 750 meters in both primary and secondary Malaysia lowland and hill forests (Jantan and Razak, 1990).

A significant number of research studies have been conducted on *Aquilaria malaccensis, Aquilaria hirta* or *Aquilaria rostrata* (Ng et al., 1997). There is very little information on the quality of the different gaharu produced. The most popular species generally associated with gaharu is *Aquilaria malaccensis.* This species is synonymous with *Aquilaria agallocha* from India (Chang, 2002).

Table 2.1: Gaharu producing species of *Aquilaria* in Peninsular Malaysia

Species	Local name for resinous wood	Grade
A. malaccensis	Gaharu	Medium
A. microcarpa	Garu	-
A. hirta	Chandan	Medium
A. rostrata	-	-
A. beccariana	Gaharu, tanduk	-

Source : Chang (2002)

2.1.2 Chemical Components of Gaharu

Generally, gaharu oils are mixture of sesquiterpenes, sesquiterpene alcohols, oxygenated compounds, chromone derivatives and resin. The most important compounds in the gaharu oils are agarospirol, jinkohol-eremol, jinkohol and kusenol that may contribute to the characteristic aroma of gaharu. Other compounds such as 2-(2-4'-methixyphenylethyl) chromone produce a long lasting fragrance upon burning.

2.1.2.1 Agarospirol

Agarospirol is one of the important compounds that contribute to the special aroma of gaharu essential oil. The IUPAC name for Agarospirol is 2-(6,10-dimethyl-2-spiro[4.5]dec-9-enyl)propan-2-ol. The formula molecule for Agarospirol is $C_{15}H_{26}O$. This compound has a molecular weight of 222.366g/mol. The functional groups in agarospirol are hydroxyl and alkenes. The chemical structure of agarospirol is aromatic as shown in Figure 2.6.

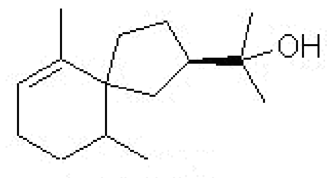

Figure 2.6: The chemical structure of agarospirol

2.1.2.2 Jinkoh-eremol

The IUPAC name for jinkoh-eremol is 2-(8,8a-dimethyl-2,3,4,6,7,8-hexahydro-1H-napthalen-2-yl)propan-2-ol. The formula molecule for Jinkoh-eremol is $C_{15}H_{26}O$. This compound has a molecular weight of 222.366g/mol. The functional groups in Jinkoh-eremol are hydroxyl and alkene. The chemical structure of Jinkoh-eremol is aromatic as shown in Figure 2.7.

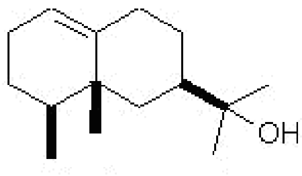

Figure 2.7: The chemical structure of jinkoh-eremol

2.1.2.3 Khusenol

The IUPAC name for khusenol is 2-(2,4-dihydroxyphenyl)-3,7-dihydroxy-8-(5-hydroxy-5-5methyl-2-prop-1-en-en-2-yl-hexyl)-5-methoxy-chroman-4-one. The formula molecule for khusenol is $C_{26}H_{32}O_8$. This compound has a molecular weight of 472.527 g/mol. The functional groups in khusenol are hydroxyl, alkene, ether and ester. The chemical structure of khusenol is aromatic as shown in Figure 2.8.

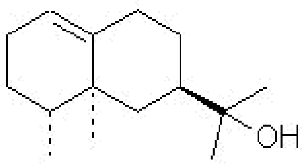

Figure 2.8: The structure of khusenol

2.1.3 Harvesting of Gaharu

Two methods of collecting gaharu from the infected gaharu trees were observed. The first was simply to cut down the infected tree, slice off the bark and sapwood of the trunk or even the roots of the tree at the place which was suspected to contain gaharu. The crude gaharu was then collected and finely sliced. This technique is commonly applied by gaharu collectors in Sumatra and Kalimantan. The second collecting technique (called tubuk) was widely used by Punan and Kenyah ethnic groups in East Kalimantan. It involved slicing off part of the trunk of the infected tree without necessarily cutting down the tree. Slicing and chopping was affected up to the nearest part of the inner core of the wood leaving the main part of the trunk intact. The chopped woods collected from the trunk were again sliced for preparation of commercial gaharu (Soehartono and Mardiastuti, 1997).

2.1.4 Market of Gaharu

The majority of gaharu harvested is exported, only small quantities being used locally, primarily for the production of incense. The main forms of gaharu in trade in Malaysia are wood types such as flakes, chips, incense and occasionally powder. Besides that, finished products such as perfume are also traded. On the other hand, non-resinous wood harvested from gaharu tree is categorized as a light hardwood that is not durable and is easily stained by fungal growth, hence it is not a popular trade material.

Demand for gaharu currently far exceeds the available supply, which is naturally restricted owing to the nature of its formation. Gaharu is only found in a small percentage of gaharu trees of those species known to produce it. The high value of gaharu products is also stimulating illegal harvest and trade in several range countries.

2.2 The Principles of Solid Liquid Extraction

Solid liquid extraction is one of the methods for preserving valuable resources in addition to protecting the environment from hazardous waste. Solid-liquid extraction is among the most commonly employed methods of separation, which appears in many industrial processes for example pharmaceutical industry, perfumes or pesticides manufacturing industries to recover active principles from plants (Luque de Castro and Garcia-Ayuso, 1998; Romdhane and Gourdon, 2002).

Extraction is a separation process to separate the desired solute or removed an undesired solute component from the solid phase where the solid is contacted with a liquid phase. Two phases are in intimate contact and the solutes can diffuse from the solid to the liquid phase, which causes a separation of the component originally in the solid. The extraction process also depends on how fast the compound will dissolve and reach the equilibrium concentration in the liquid. Solid-liquid extraction also known by variety of other names, such as leaching, washing, percolation, digestion, steeping, lixiviation and infusion but of this only one term, leaching has widespread use (Geankoplis, 1993; Ruthven, 1997; Luque de Castro and Garcia-Ayuso, 1998; Cacace and Mazza, 2003).

Leaching is widely used as a separation process for the following (Phipps and Eardly, 1982; Mizubuti et al., 2001; Dickey et al., 2002; Xuejun Pan et al., 2003):

i) Extraction of edible oils from seeds, beans, nuts, rice bran, wheat germ, coconut and other sources.
ii) Extraction of essential oils from flowers, leaves, wood and seeds.
iii) Extraction of oleoresins from spices.
iv) Extraction from coffee from coffee beans.
v) Extraction of oil fish from fish meal.
vi) Extraction of active ingredients from leaves, pods, seeds, flowers and barks e.g., extraction of tocopherols.
vii) Extraction of copper sulphate from copper ore.

The simplest extraction system comprises three components:

i) Solute, or the material to be extracted

ii) Solvents, which may be a liquid or a supercritical fluid at process conditions

iii) Carrier or non solute portion of the feed mixture to be separated.

For the case of countercurrent extraction and a light solvent, the flow of the materials is as shown in Figure 2.9.

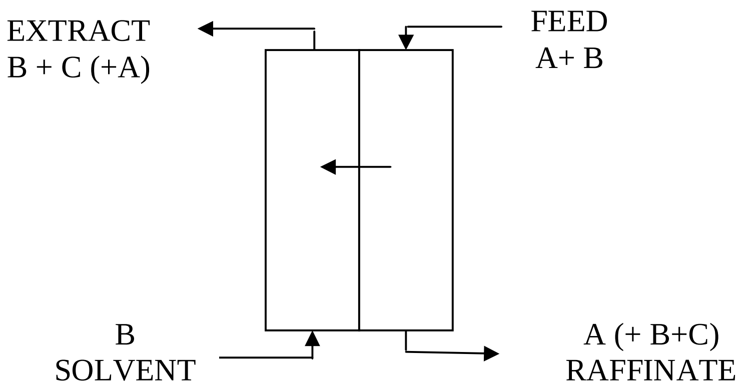

Figure 2.9: Extraction Notation

Where,

Reffinate phase: feed stream minus extracted material

Extract phase: solvent stream plus extracted material

A = Carrier, B = Solvent, C = Solute

For such system the carrier and the solvent are essentially immiscible, while the carrier-solute and solvent-solute pairs are miscible.